

AMENDMENT AND RESPONSE UNDER 37 CFR § 1.111

Serial Number: 09/464,158

Filing Date: December 16, 1999

Title: ASSAY FOR CARBOHYDRATE-FREE TRANSFERRIN

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Dkt: 697.011US1

The paragraph beginning at page 16, line 21, is amended as follows:

Separations based on centrifugation and/or filtration are convenient. In a preferred embodiment a centrifuge tube ([eg.] e.g., Eppendorf tube) and "filter cup" format may be used, and such formats are readily commercially available, for example from [Millepore] Millipore. Thus the sample and carbohydrate-binding ligand may be added to the cup in the tube and allowed to bind. The tube (and cup) is then spun, and the non-binding supernatant collects in the tube. The carbohydrate binding ligand may be such as to induce precipitation of the bound carbohydrate moieties or it may be [immobilised] immobilized, for example as a slurry [eg.] , e.g., a gel or on particles. In either case, the bound carbohydrate binding fraction is retained in the cup.

The paragraph beginning at page 24, section c., is amended as follows:

c. The suspensions are transferred to [Ultra-free] ULTRA-FREE MC Millipore UFC3 OHV (0.45 µm) filter cups and centrifuged.

The paragraph beginning at page 25, line 8, is amended as follows:

d. The suspensions are transferred to [Ultra-free] ULTRA-FREE MC Millipore UFC3 OHV (0.45 µm) filter cups and centrifuged.

The paragraph beginning at page 25, in Example 3, section b., is amended as follows

b. 0.5 ml of agarose ([Reacti-Gel] REACTI-GEL from Pierce Chemical Company, US) with lectin from *Helicobacter communis* isolated according to Lelwala et al., "Isolation of a sialic acid-specific surface haemagglutinin of *Helicobacter pylori* strain NCTC11637", Zblatt 280:93-196, 1993 [immobilised] immobilized on the agarose according to the manufacturer of the "[Reacti-Gel] REACTI-GEL " package insert, suspended in a buffer of 20 W TRIS buffer pH = 7.5 comprising 150 mM sodium chloride, and then mixed with each of the serum samples in TRIS buffer (see a. above).

The paragraph beginning at page 25, Example 3, section c., is amended as follows:

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c. The suspensions are transferred to [Ultra-free] ULTRA-FREE MC Millipore UFC3 OHV (0.45 µm) filter cups and centrifuged.

The paragraph beginning at page 27, line 11 is amended as follows:

c. The suspensions are transferred to [Ultra-free] ULTRA-FREE MC Millipore UFC3 OHV (0.45 µm) filter cups and centrifuged.

The paragraph beginning at page 27, line 22, is amended as follows:

20 µl of serum sample is mixed with 0.5 ml of a 25% preswollen [Whatman] WHATMAN QA52 anion exchange resin suspended in 20M bis(2-hydroxy)amino-tris(hydroxyethyl)methane pH = 6.3. The chloride content of the medium is carefully adjusted to separate the desired isotransferrin fractions, and this may be monitored by HPLC or isoelectric [focussing] focusing. Thereafter, 0.5ml agarose elderberry bark lectin (Vector Laboratories) is added, and the suspension is mixed gently. The suspension is thereafter filtered by centrifugation in a Millipore [Ultra-Free] ULTRA-FREE MC UFC3 OHV filter cup, and the filtrate is collected. 200 µl of the filtrate is mixed with 200 µl of an transferrin antibody (Dako) solution diluted 1:10 in 0.27M TRIS, 4.5% PEG 8000, 4.3 W sodium azide pH = 7.4, and the nephelometric signal is read and interpolated in a standard curve constructed from standards of known concentration of human transferrin.

The paragraph beginning at page 28, Example 7, section c., is amended as follows:

c. The suspensions are transferred to [Ultra-free] ULTRA-FREE MC Millipore UFC3 OHV (0.45 µm) filter cups and centrifuged.

IN THE CLAIMS

Please substitute the claim set in entitled "Clean Version of Pending Claims", attached hereto, for the previously pending claim set. The substitute claim set is intended to reflect the amendment of previously pending claims 1-5. The specific amendments to individual claims are detailed in the following marked-up claims.